UNIVERSITI PUTRA MALAYSIA

CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *MICROMELUM MINUTUM* (RUTACEAE) AND TWO *EUGENIA* SPECIES (MYRTACEAE)

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By

RATNA ASMAH SUSIDARTI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

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To all my family,

Alma maters,

and Ibu Pertiwi……Indonesia

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of requirement for the degree of Doctor of Philosophy

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Chairman: Prof. Dr. Mawardi Rahmani

Faculty : Science and Environmental Studies

Drug discovery has been a goal of mankind since prehistoric times. Plants continue to be an exceptionally viable source of biologically active natural products. However, only small portion of the higher plants has been phytochemically and pharmacologically investigated.

Two separate collections of *Micromelum minutum* from Sabah and two *Eugenia* species, *E. chlorantha* and *E. cumingiana* collected from the northern forest of Perak were subjected to phytochemical investigation with the isolation of a number of compounds. The structures of these compounds were elucidated by using spectroscopic methods such as IR, UV, NMR, MS and also by comparison with previous reports. The crude extracts, essential oil and isolated compounds

from these plant materials were evaluated for their antiinflammatory, cytotoxic and antimicrobial activities using carrageenan-induced rat paw oedema, MTT and disc diffusion methods, respectively. Some isolated compounds showed interesting biological activities.

The investigation of the leaves of *M. minutum* from first collection afforded two known triterpenes, 5(6)-gluten-3-one **(76)** and 5(6)-gluten-3α-ol **(77)**, β-sitosterol **(41)** and five new coumarins, 3″,4″-dihydrocapnolactone **(78)**, 2′,3′-epoxyisocapnolactone **(79)**, 8-hydroxyisocapnolactone-2′,3′-diol **(80)**, 8-hydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol **(81)** and 8,4″-dihydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol **(82)**. The first three coumarins together with a known coumarin, 8-methoxycapnolactone **(83)** and stigmasterol **(84)** were also found to be the constituents of the leaves of another separate collection of the same species. The compounds 5(6)-gluten-3-one **(76)**, 5(6)-gluten-3α-ol **(77)** and 8-methoxycapnolactone **(83)** were new for the genus *Micromelum*. Extraction and separation of the stem barks of both collections only gave β-sitosterol **(41)**.

The two new coumarins, 2′,3′-epoxyisocapnolactone **(79)** and 8-hydroxy-isocapnolactone-2′,3′-diol **(80)** were significantly toxic to T-lymphoblastic leukemia (CEM-SS) and promyelocytic leukemia (HL60) cell lines. The IC_{50} value of the former against these two cancers cell lines were 3.9 and 4.2 μg/ml, while those of the latter were 2.9 and 2.5 μg/ml, respectively. However, both compounds showed moderate activity (IC_{50} 5.0-10.0 μg/ml) towards cervical

cancer (HeLa) and liver cancer (HepG2) cell lines. The cytotoxic activity of these compounds against normal mouse fibroblast (3T3) cell line was weak. The antibacterial s creening r esult showed that these two coumarins were also active against human and shrimp pathogenic bacteria. Another new coumarin, 3″,4″-dihydrocapnolactone **(78)** possessed weak cytotoxic and antibacterial activity, while the most polar coumarin, 8,4″-dihydroxy-3″,4″-dihydrocapnolactone-2′,3′-diol **(82)** was not active.

Extraction and separation of the leaves and stem bark of *E. chlorantha* yielded friedelin **(43)** which possessed moderate cytotoxic activity (IC_{50} of 5.8 μg/ml). The essential oil of leaves of this plant constituted principally oxygenated sesquiterpenes and showed weak cytotoxic and antibacterial activities. The methanol and aqueous extracts of the leaves possessed antiinflammatory activity, but they performed relatively lower activity than indomethacin. From the stem bark of *E. cumingiana*, β-sitosterol **(41)** and two unidentified compounds w ere isolated. In addition, all the extracts, essential oil and isolated compounds from the plants studied were inactive towards the target fungi.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan untuk ijazah Doktor Falsafah

KANDUNGAN KIMIA DAN AKTIVITI BIOLOGI DARIPADA *MICROMELUM MINUTUM* (RUTACEAE) DAN DUA SPESIES *EUGENIA* (MYRTACEAE)

Oleh

RATNA ASMAH SUSIDARTI

Mac 2003

Pengerusi: Prof. Dr. Mawardi Rahmani

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Sejak zaman dahulu lagi penemuan ubat-ubatan telah menjadi matlamat manusia. Tumbuh-tumbuhan berterusan menjadi sumber yang penting bagi hasilan semulajadi yang mempunyai kesan aktif biologi. Namun hanya sebahagian kecil daripadanya telah dikaji secara fitokimia dan farmakologi.

Kajian fitokomia ke atas dua pengumpulan berasingan *Micromelum minutum* dari Sabah dan dua spesies *Eugenia*, *E. chlorantha* dan *E. cumingiana* yang dikumpulkan dari kawasan hutan di sebelah utara Perak telah dijalankan dengan pemencilan beberapa sebatian. Struktur dari sebatian-sebatian ini dikenal pasti dengan menggunakan kaedah spektroskopi seperti IM, UL, RMN, SJ dan juga perbandingan dengan kajian-kajian yang lepas. Ekstrak mentah, minyak pati dan sebatian-sebatian yang dipencilkan dari bahagian tertentu tumbuhan ini diuji

untuk aktiviti antiinflamasi menggunakan kaedah pembengkakan kaki tikus yang diinduksi "carrageenan", sitotoksik dengan kaedah MTT dan antimikrob dengan kaedah peresapan cakera. Beberapa sebatian yang dipencilkan menunjukkan aktiviti biologi yang menarik.

Kajian ke atas daun *M. minutum* (pengumpulan pertama) telah menghasilkan dua triterpena yang telah dikenali, 5(6)-gluten-3-on **(76)** dan 5(6)-gluten-3α-ol **(77)**, β-sitosterol **(41)** dan lima koumarin baru, 3″,4″-dihidrokapnolakton **(78)**, 2′,3′-epoksisokapnolakton **(79)**, 8-hidroksisokapnolakton-2′,3′-diol **(80)**, 8-hidroksi-3″,4″-dihidrokapnolakton-2′,3′-diol **(81)** dan 8,4″-dihidroksi-3″,4″-dihidrokapnolakton-2′,3′-diol **(82)**, Tiga koumarin yang pertama bersama-sama dengan satu koumarin yang telah dikenali, 8-metoksikapnolakton **(83)** dan stigmasterol **(84)** juga didapati daripada daun pengumpulan berasingan kedua dari spesies yang sama. Sebatian-sebatian 5(6)-gluten-3-on **(76)**, 5(6)-gluten-3α-ol **(77)** dan 8-metoksikapnolakton **(83)** adalah baru bagi genus *Micromelum*. Hasil dari pengekstrakan dan pengasingan kulit batang dari kedua-dua pengumpulan berasingan hanya menghasilkan β-sitosterol **(41)**.

Dua koumarin yang baru, 2′,3′-epoksisokapnolakton **(79)** dan 8-hidroksisokapnolakton-2′,3′-diol **(80)** menunjukkan kesan toksik yang signifikan terhadap sel T-limpoblastik leukemia (CEM-SS) dan promielositik leukemia (HL60). Nilai IC_{50} daripada koumarin yang pertama terhadap dua sel kanser ini

masing-masing adalah 3.9 and 4.2 μg/ml, manakala koumarin yang kedua mempunyai IC_{50} masing-masing 2.9 and 2.5 μg/ml. Meskipun demikian, kedua sebatian ini hanya menunjukkan aktiviti yang sederhana (IC_{50} 5.0-10.0 μg/ml) terhadap sel kanser servik (HeLa) and hati (HepG2). Aktiviti sitotoksik daripada kedua-dua koumarin terhadap sel fibroblast tikus yang normal adalah rendah. Hasil biocerakinan antibakteria menunjukkan bahwa kedua-dua koumarin ini juga aktif terhadap bakteria yang patogenik kepada manusia dan udang. Koumarin 3″,4″-dihidrokapnolakton **(78)** mempunyai aktiviti sitotoksik dan antibakteria yang lemah, manakala koumarin yang paling polar, 8,4″-dihidroksi-3″,4″-dihidrokapnolakton-2′,3′-diol **(82)** didapati tidak mempunyai sebarang aktiviti sitotoksik dan antibakteria.

Pengekstrakan dan pengasingan bagi daun dan kulit batang *E. chlorantha* menghasilkan friedelin **(43)** yang mempunyai aktiviti sitotoksik sederhana (IC_{50} 5.8 μg/ml). Ekstrak minyak pati daripada daun tumbuhan ini mengandungi terutamanya seskuiterpena teroksigenasi dan menunjukkan aktiviti sitotoksik dan antibakteria yang lemah. Ekstrak methanol dan akueus daripada daun mempunyai aktiviti antiinflammasi yang lebih rendah daripada indomethacin. Dari kulit batang *E. cumingiana* telah dipencilkan β-sitosterol **(41)** dan dua sebatian yang tidak dikenali. Semua ekstrak mentah, minyak pati dan sebatian-sebatian yang telah dipencilkan daripada tumbuh-tumbuhan yang dikaji didapati tidak aktif terhadap kulat.

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I certify that an Examination Committee met on 26 March 2003 to conduct the final examination of Ratna Asmah Susidarti on her Doctor of Philosophy thesis entitled "Chemical Constituents and Biological Activities of *Micromelum minutum* (Rutaceae) and Two *Eugenia* species (Myrtaceae)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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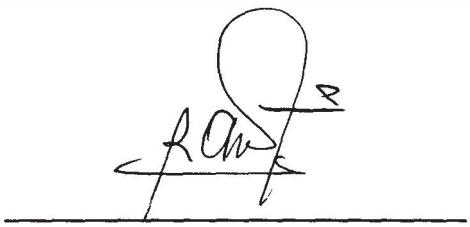
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.



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